ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 1983, p. 409–412
0066-4804/83/090409-04$02.00/0

Vol. 24, No. 3

In Vitro Antibacterial Activity of Concentrated Polyethylene Glycol 400 Solutions

JORGE CHIRIFE,[1]* LEÓN HERSZAGE,[2] ARABELLA JOSEPH,[3] JUAN P. BOZZINI,[3] NÉLIDA LEARDINI,[3] AND ELISA S. KOHN[3]

Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, 1428 Buenos Aires[1]; Viamonte 1620, 2° C, 1055 Buenos Aires[2]; and Instituto Nacional de Microbiologia "Dr. Carlos G. Malbrán," Buenos Aires,[3] Argentina

Received 13 April 1983/Accepted 21 June 1983

It was found that concentrated polyethylene glycol 400 (PEG 400) solutions have significant antibacterial activity against various pathogenic bacteria, including *Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli*, and *Staphylococcus aureus*. This effect might be attributed to two effects: lowering of water activity and, superimposed on this, the specific action of PEG-400 molecules on bacterial cells. Phase-contrast microscopic observations of cells placed in contact with PEG 400 revealed clumping and morphological changes of bacterial cells. The larger changes in appearance were evidenced by the species which were more rapidly killed by PEG 400. The results obtained suggested that concentrated PEG 400 solutions may have a potential value in medicine as a topical antibacterial agent. The feasibility of this application is the subject of present investigation.

In the past years, various studies have been made on the relation of microorganisms to high solute concentrations. These have included studies of growth inhibition mechanisms, the solute concentration needed to inhibit growth, and the physiological basis of microorganisms that can withstand such high solute concentrations (1, 3, 9, 10). It has been observed that the water requirements of bacteria as well of other microorganisms are best expressed in terms of water activity (a_w) rather than water concentration. Water activity is given as $a_w = p/p_0$ where p is the vapor pressure of water in solution and p_0 is the vapor pressure of pure water at the same temperature. At present, numerous data are available on the relation between a_w and the ability of microorganisms to grow (9). However, it has also established that the a_w of the medium is not the only determining factor regulating the biological response of bacteria; the nature of the solute used to reduce a_w is also important (5).

The bactericidal activity of a variety of glycols has been studied by Robertson et al. (14). They investigated the bactericidal action in vitro of a number of glycols for pneumococci, hemolytic streptococci, and staphylococci. Three glycols were studied more extensively than the others, namely, propylene, dipropylene, and triethylene. Olitzky (12) also reported on the germicidal efficiency of concentrated propylene glycol solutions. Plitman et al. (13) investigated the bacteriostatic and bactericidal activity of several diols, employing *Staphylococcus aureus* as test organism.

Recently, Vaamonde et al. (15) showed that polyethylene glycol 400 (PEG 400) appeared to have a significant inhibitory effect on one strain of *S. aureus*, independent of a_w lowering. The present study describes investigations that were carried out to explore the bactericidal effect of concentrated PEG 400 against various pathogenic bacteria relevant to infected wounds and other superficial lesions. The bacterial species studied included *S. aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Escherichia coli*. To our knowledge, no detailed study of the bactericidal activity of concentrated PEG 400 solutions on these bacteria has been reported.

MATERIALS AND METHODS

Microorganisms. The sources of the strains used in this study and their strain numbers are as follows: *S. aureus* ATCC 6538P; *P. aeruginosa* ATCC 27853; *K. pneumoniae* ATCC 10031; *E. coli* ATCC 25922; *S. aureus* 41/82, a wild-type strain obtained from Hospital Muñiz, Buenos Aires, Argentina; *P. aeruginosa* 15/4, *K. pneumoniae* 6440, and *E. coli* 11197, strains obtained from the culture collection of Instituto Nacional de Microbiología "C. G. Malbrán".

Media. Brain heart infusion (BHI) broth (Oxoid Ltd., London, England) was used for all tests. PEG 400 (Mallinckrodt Chemical Works, St. Louis, Mo.) was added to BHI before sterilization. After dissolution of PEG 400, the pH was adjusted to 7.0 by the addition of 8 N HCl. The media was autoclaved with precautions to avoid loss of water by vaporization which could change the concentration of PEG 400. In most experiments the concentration of PEG 400 in the media was adjusted to 1.6 g of PEG 400 per g of water;

in some experiments a concentration of 4.8 g of PEG 400 per g of water was utilized.

Technique of tests. Growth inhibition studies were made in 250-ml screw-top glass bottles containing about 18 g of inoculated medium containing PEG 400 which were incubated at 35°C in a constant-temperature cabinet. The bacteria used for the inoculum were derived from a culture in the log phase.

Enumeration procedure. Counts were determined by the use of plate count agar (Difco Laboratories, Detroit, Mich.) for *S. aureus*, *K. pneumoniae*, and *E. coli*; CLED medium with Andrade indicator (Oxoid Ltd.) was used for *P. aeruginosa*. The samples were serially diluted (1:10) with 0.1% peptone (Oxoid Ltd.) before plating; the volume plated was 0.1 ml. The plates were incubated at 35°C for 24 to 48 h, and the colonies were counted.

Phase-contrast microscopy. Observations were carried out at room temperature with a dark contrast-phase Nikon Labophot microscope. A CF acromat 100/1.25 DLL objective and a CF Photo ocular 5× projective (field number 20) were used as an optical recording combination. Photomicrographs (at magnifications of 500 on the negative) were taken with a Nikkon FX Microflex 35-mm manual camera on Ilford PAN F film exposed as 18 DIN (50 ASA).

Cells to be observed and photographed were obtained from BHI broth cultures in logarithmic growth phase which were centrifuged at 20 × *g*. The pellets were resuspended in fresh culture medium with PEG 400 by using a vortex mixer. The amount of former medium (about 0.2 ml) was taken into account to obtain the final PEG 400 concentrations as well as for the determination of the number of cells per milliliter useful for microscope sampling observations (about 10^6 to 10^8 cells per ml). Samples were removed from media and placed on microscope slides with a calibrated loop (10^{-3} ml) at various times after the cells were subjected to PEG 400 solutions (1.6 g of PEG 400 per g of water in broth).

Water activity determination. The water activity of media containing PEG 400 was determined by using a fiber-dimensional hygrometer, a_w-Wert Messer, manufactured by Firma Lufft, Stutgart, West Germany (2).

RESULTS

Survival of colony-forming ability. Figure 1 shows the behavior of *S. aureus* ATCC 6538 P, *E. coli* ATCC 25922, and *K. pneumoniae* ATCC 10031 in BHI media with PEG 400 (1.6 g of PEG 400 per g of water in medium). Initial inoculum levels of all microorganisms were fixed at 10^7 to 10^8 CFU/ml. It can be seen that viable cells of *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 10031 declined rapidly, leading to almost total loss of viable population in 10 to 14 h of incubation. *S. aureus* ATCC 6538 P was far more resistant than the other bacteria studied, although the viable population also declined continuously; almost total destruction of cells was achieved after about 120 h of incubation. Results similar to those in Fig. 1 were obtained with *E. coli* 11179, *K. pneumoniae* 6440, and *S. aureus* 41/82. It is noteworthy that control experiments

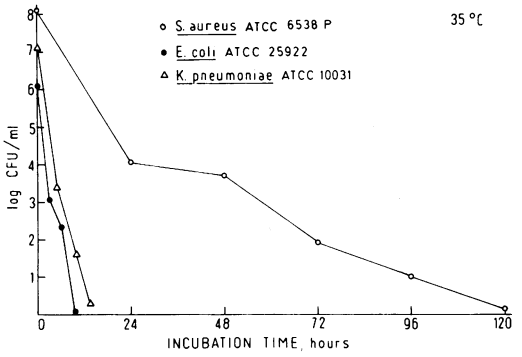

FIG. 1. Survival of colony-forming ability of *S. aureus* ATCC 6538 P, *E. coli* ATCC 25922, and *K. pneumoniae* ATCC 10031 in PEG 400 added to BHI medium (1.6 g of PEG 400 per g of water in broth) incubated at 35°C.

(BHI broth without PEG 400) were also performed (not shown) and showed that all species remained at 10^8 to 10^9 CFU/ml during the period of incubation.

Figure 2 shows the behavior of *P. aeruginosa* ATCC 27853 and 15/4 in BHI broth with PEG 400 added to the same level of concentration as before. It can be seen that PEG 400 kills the cells rapidly as compared to other species studied; almost complete inactivation of cells occurred at 2 to 4 h of incubation.

Additional survival experiments were performed by using a more concentrated solution of PEG 400; in this case a value of 4.8 g of PEG 400 per g of water in medium was adopted. It was found that complete destruction of cells of *P. aeruginosa* ATCC 27853 (initial inoculum, 3.8 × 10^8 CFU/ml), *P. aeruginosa* 15-4 (initial inoculum, 6.8 × 10^8 CFU/ml), and *E. coli* 11197 (initial inoculum, 3.7 × 10^8 CFU/ml) occurred in

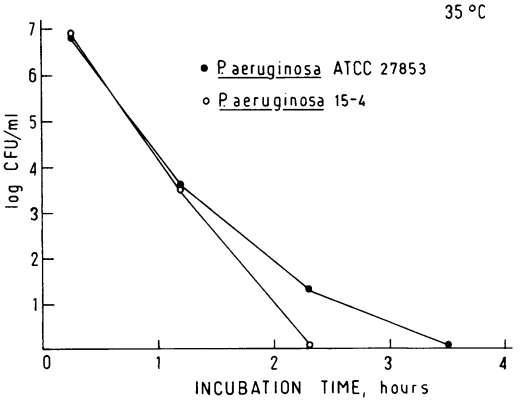

FIG. 2. Survival of colony-forming ability of *P. aeruginosa* ATCC 27853 and 15/4 in PEG 400 added to BHI medium (1.6 g of PEG 400 per g of water in broth) incubated at 35°C.

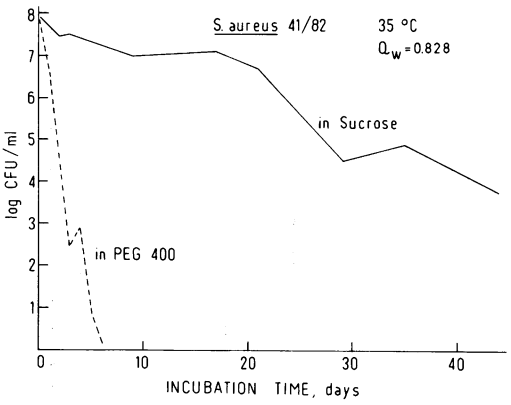

FIG. 3. Comparison of survival curves of *S. aureus* 41/82 in BHI broth of a_w lowered by the addition of PEG 400 or sucrose and incubated at 35°C.

only 20 to 25 min of incubation at 35°C.

An attempt was made to distinguish between the effects of lowered a_w and specific solute effects on bacteria, as follows. Figure 3 compares the survival curves of *S. aureus* 41/82 in BHI broth with the a_w lowered to the same value (around 0.828) by the addition of either PEG 400 or sucrose (220 g of sucrose per 100 g of water); data for the sucrose solution were taken from Chirife et al. (4). Sucrose was used for comparison since it is also a nonionic solute of similar molecular weight. It can be seen that the bactericidal action of PEG 400 is dramatically different (faster) than that of sucrose. Similar results (not shown) were obtained when comparing the antibacterial activities of PEG 400 and sucrose against *E. coli*, *P. aeruginosa*, or *K. pneumoniae* in solutions of equal a_w.

Microscopic observations. Cells of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus* were observed under a phase-contrast microscope 1 to 2 min after being placed in contact with PEG 400 (1.6 g of PEG 400 per g of water in medium); in some cases the observations were made after 15 min. The phase-contrast microscopic observations indicated that in all cases PEG 400 caused immediate clumping of the cells. This is illustrated in Fig. 4, which shows normal log-phase cells of *K. pneumoniae* 6440 (Fig. 4A) and the same cells shortly after being placed in broth with PEG 400 (Fig. 4B). Similar morphological changes were observed for the other bacteria studied. It is noteworthy, however, that in the case of *S. aureus* the morphological modifications (clump formation) were less evident than for the other cells.

DISCUSSION

It is generally accepted that when a solute such as sucrose is used to reduce the a_w, the a_w itself is the main determining factor regulating the biological response of bacteria. Bacterial cells are largely impermeable to sucrose (8), and lowered a_w withdraws water from the cell and may cause plasmolysis. However, it is apparent from the results in Fig. 3 that PEG 400 produces two effects: lowering of a_w and, superimposed

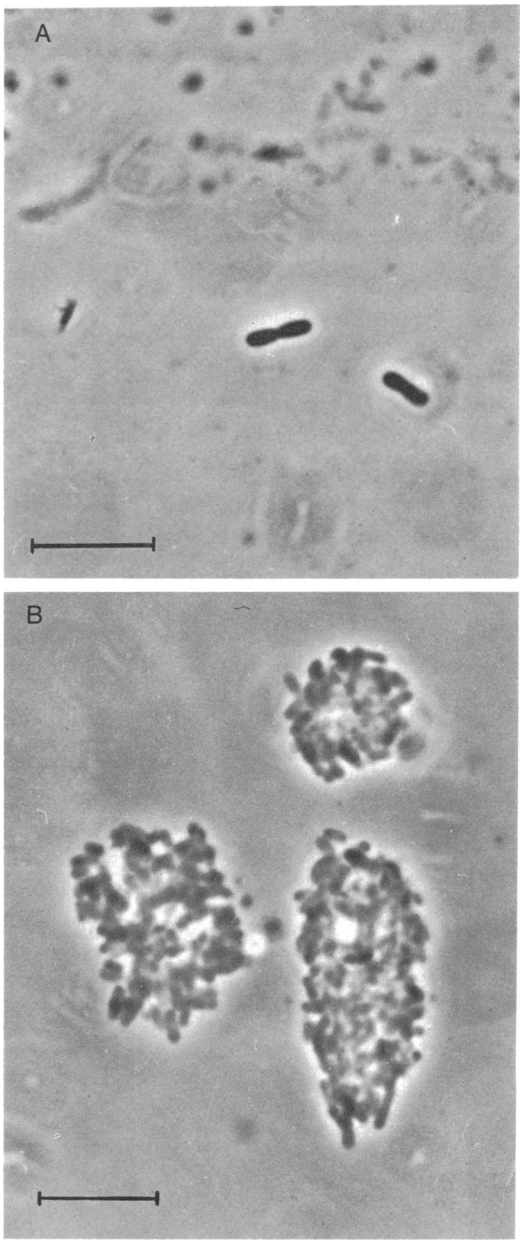

FIG. 4. Phase-contrast micrographs of cells of *K. pneumoniae* ATCC 10031. (A) Normal log-phase cells; (B) log-phase cells shortly after being placed in broth with PEG 400. Bar, 10 μm.

on this, the specific action of its molecules on bacterial cells.

PEG 400 appears to cause drastic changes in cell morphology within minutes after addition to the cells. However, we cannot say that these effects (clumping) are directly related to the decrease in bacterial count in the PEG 400-containing cultures due to the relatively slow effect observed on bacterial survival (Fig. 1). We observed, however, that the larger changes in morphology were evidenced by the species which were more rapidly killed by PEG 400, i.e., *K. pneumoniae* and *P. aeruginosa*. As previously mentioned, clumping of the cells was less conspicuous in *S. aureus* (Fig. 1), which was far more resistant than the other bacteria.

The results obtained here suggest that concentrated PEG 400 solutions may have a potential value in medicine as a topical antibacterial agent. Further studies are under way on the rate at which killing of bacteria could be produced by more concentrated solutions, i.e., above 4.8 g of PEG 400 per g of water, as well as on the activity of PEG 400 in the presence of serum or albumin. Information on these latter points is of particular importance in relation to the effectiveness of PEG 400 as a disinfecting agent, since we are interested in a compound which is rapidly bactericidal. Of course, the killing effect of PEG 400 is much less than that of traditional disinfecting agents. However, its merits should be judged, considering that PEG 400 meets many of the physicochemical requirements for an ideal disinfectant for topical use. PEG 400 is neutral, odorless, highly soluble in water (soluble in all proportions), and nonirritating to the skin, does not decompose, has a low vapor pressure, and its toxicity from acute oral administration or topical application is low (6, 11). At present PEG 400 (as well as other polyethylene glycols) has several pharmaceutical applications not due to its antibacterial properties. Polyethylene glycols find their chief application in the preparation of hydrophylic ointment bases incorporating a wide variety of therapeutic materials, including antibacterials, antibiotics, and steroids. PEG 400 may be also safely used in foods as a coating, binder, or plasticizing or bodying agent (7).

ACKNOWLEDGMENTS

We acknowledge financial support from the Subsecretaría de Ciencia y Technología de la República Argentina (Programa Nacional de Tecnología de Alimentos) and from the World Sugar Research Organization.

LITERATURE CITED

1. **Brown, A. D.** 1976. Microbial water stress. Bacteriol. Rev. **40**:803–846.
2. **Chirife, J., and C. Ferro Fontán.** 1980. The prediction of water activity in aqueous solutions in connection with intermediate moisture foods. V. Experimental investigation of the a_w lowering behavior of sodium lactate and some related compounds. J. Food Sci. **45**:802–804.
3. **Chirife, J., C. Ferro Fontán, and O. C. Scorza.** 1981. The intracellular water activity of bacteria in relation to the water activity of the growth medium. J. Appl. Bacteriol. **50**:475–479.
4. **Chirife, J., L. Herszage, A. Joseph, and E. S. Kohn.** 1983. In vitro study of bacterial growth inhibition in concentrated sugar solutions: microbiological basis for the use of sugar in the treatment of infected wounds. Antimicrob. Agents Chemother. **23**:766–773.
5. **Christian, J. H. B.** 1981. Specific solute effects on microbial water relations, p. 825–854. *In* L. B. Rockland and G. F. Stewart (ed.), Water activity: influences on food quality. Academic Press, Inc., New York.
6. **Dow Chemical U.S.A.** 1974. Polyethylene glycols handbook. Dow Chemical Co., Midland, Mich.
7. **Furia, T. E.** 1975. Handbook of food additives, 2nd ed. CRC Press, Cleveland, Ohio.
8. **Gould, G. W., and J. C. Measures.** 1977. Water relations in single cells. Philos. Trans. R. Soc. London Ser. B. **278**:151–166.
9. **Kushner, D. J.** 1971. Influence of solutes and ions on microorganisms, p. 259–283. *In* W. B. Hugo (ed.), Inhibition and destruction of the microbial cell. Academic Press, Inc., London.
10. **Kushner, D. J.** 1978. Life in high salt and solute concentrations: halophilic bacteria, p. 317–368. *In* D. J. Kushner (ed.), Microbial life in extreme environments. Academic Press, Inc., New York.
11. **Lockard, J. S., R. H. Levy, W. C. Congdom, and L. L. DuCharme.** 1979. Efficacy and toxicity of the solvent polyethylene glycol 400 in monkey model. Epilepsia **20**:77–84.
12. **Olitzky, I.** 1965. Antimicrobial properties of a propylene glycol based topical therapeutic agent. J. Pharm. Sci. **54**:787–788.
13. **Plitman, M., Y. Park, R. Gómez, and A. J. Sinskey.** 1973. Viability of *Staphylococcus aureus* in intermediate moisture meats. J. Food Sci. **38**:1004–1008.
14. **Robertson, O. H., E. M. Appel, T. T. Puck, H. M. Lemon, and M. H. Ritter.** 1948. A study of the bactericidal activity in vitro of certain glycols and closely related compounds. J. Infect. Dis. **83**:124–137.
15. **Vaamonde, G., J. Chirife, and O. C. Scorza.** 1982. An examination of the minimal water activity for *Staphylococcus aureus* ATCC 6538 P growth in laboratory media adjusted with less conventional solutes. J. Food Sci. **47**:1259–1262.